Mycotoxins: Of Molds & Maladies


Aspergillus
Photo:A RS/USDA

From the beginning of organized crop production through present-day agriculture, mycotoxins—toxic metabolites produced by fungi—have presented health risks to both human and animal populations. Mycotoxins are secondary metabolites produced by certain molds that infect food crops in the field and during storage. Depending on the quantities produced and consumed, mycotoxins can cause acute or chronic toxicity in the animals and humans who eat contaminated crops or foods prepared from them. Health effects of mycotoxins may include immunological effects, organ-specific toxicity, cancer, and, in some cases, death. Agricultural workers are also at risk for dermal and respiratory exposures during crop harvest and storage.

Mycotoxin contamination is a worldwide problem affecting staple crops such as corn (maize) and small grains (such as wheat), as well as tree nuts, peanuts, sorghum, and many others. Many countries regulate the maximum allowable concentrations of specific mycotoxins in food commodities and animal feed. Until recently, dietary and occupational exposures were the primary areas of concern, but with growing attention being paid to the problems associated with indoor molds and respiratory exposures, researchers are recognizing that the potential scope of mycotoxin exposures is broader than originally suspected. This scope now includes inner-city environments, with a special focus on older or poorly maintained structures that are more susceptible to harboring molds.

Researchers are also recognizing that specific groups within a population may be more vulnerable to exposure than the population at large. For example, in the United States, the Hispanic community consumes a greater quantity of corn products compared to the general population. Because corn is vulnerable to contamination by several mycotoxins, it is possible that individuals within the Hispanic community are exposed to higher dietary levels of mycotoxins than the average American. This higher exposure could place them at a greater risk of health problems.

The effects of mycotoxins have been recorded for millennia in sources as diverse as 5,000-year-old Chinese texts, the Old Testament of the Bible, and numerous scientific journals. As research into these compounds and their effects has accumulated over the last several decades, mycotoxins have been revealed to be an extremely diverse group of compounds.

During the Middles Ages, periodic epidemics of St. Anthony's fire, now known as ergotism, afflicted countless individuals who had consumed moldy rye. Gangrenous ergotism, the form that inspired the medieval name, was accompanied by fiery pain, swelling, and gangrene in the limbs. Convulsive ergotism, a second form of the toxicosis, was accompanied by convulsions and hallucinations, among other symptoms. Both forms could be fatal. Research in the 1940s identified ergot alkaloids produced by *Claviceps* spp. as the mycotoxigenic source of ergotism. Ergotism has occurred very rarely in recent decades; with regard to agricultural crops, current research focuses on the toxigenic and carcinogenic potentials associated with molds including species of *Aspergillus* and *Fusarium*. With regard to indoor molds and respiratory health issues, *Stachybotrys chartarum* (formerly *S. atra*) contributes a challenging new facet to the field of mycotoxicology.

A Centerpiece of Mycotoxin Research

"Monolithic" is how John D. Groopman, a professor of environmental health sciences at the Johns Hopkins University School of Public Health in Baltimore, Maryland, describes the literature on aflatoxins—mycotoxins produced by *Aspergillus* spp. "As far as the epidemiology in people goes, the overabundance of literature covers aflatoxins," he says. "Aflatoxin levels are regulated not only by the Food and Drug Administration [FDA] and the U.S. Department of Agriculture [USDA] but also in world commerce. I don't believe that's true for almost any of the other mycotoxins." This emphasis reflects the central role of aflatoxins, especially aflatoxin B_1 (AFB_1), in mycotoxicological research since the early 1960s. Primarily produced by *A. flavus* and *A. parasiticus*, AFB_1 causes liver and kidney toxicity in several species and is most prominently known as a potent liver carcinogen in humans and animals. *Aspergillus* spp. exist worldwide and live off of a number of crops, although corn and peanuts are the most commonly contaminated commodities. *A. flavus* and *A. parasiticus* are the most common forms of *Aspergillus* and are also capable of producing other forms of aflatoxin. Frequently, there is contamination with multiple forms of aflatoxin. However, regardless of which aflatoxins are produced, AFB_1 is always among them, and it is the most toxic.

AFB_1 has been shown to be mutagenic in

many *in vitro* model systems and is a proven carcinogen in many animal species, including rodents and nonhuman primates. The primary cancer site in these studies has been the liver, but in some cases a link has been demonstrated to lung, kidney, and colon tumors. In human epidemiological studies, chronic dietary AFB_1 exposures have been strongly linked to increased incidence of liver cancer. On the basis of this information, AFB_1 is classified as a known human carcinogen by the International Agency for Research on Cancer and the National Toxicology Program.

The carcinogenic potency of AFB_1 is not manifested until it undergoes activation by the cytochrome P450 and other oxidative enzymes. These enzymes transform the aflatoxin into several products, including the genotoxic AFB_1–*exo*-8,9-epoxide. This epoxide can be shunted by glutathione *S*-transferase in the liver, but some will intercalate, or wedge itself, between DNA base pairs. In this position, the epoxide rapidly reacts with the DNA to produce an N^7-guanyl adduct. As demonstrated in several animal models, these adducts are produced in the greatest amounts in the liver, although some are also produced in the kidney or lung. Through DNA repair and chemical stability mechanisms, AFB_1–DNA adducts can be removed and excreted, but some adducts prove resistant to repair, thereby setting the stage for mutation events and carcinogenesis. The potential for AFB_1-induced liver cancer is enhanced in individuals who are also infected with the hepatitis B virus, a recognized carcinogenic virus. In AFB_1-exposed populations, examination of liver tumors reveals a high incidence of a specific *p53* tumor suppressor gene mutation. In AFB_1-exposed individuals infected with the hepatitis B virus, this mutation is associated with 50–60% of the tumors. By understanding the mechanism of action of AFB_1, we are identifying guideposts for developing intervention strategies, says Groopman.

The Depth of the Field

Not all carcinogenic mycotoxins act through a genotoxic mechanism, and the fumonisins provide a case in point. The fumonisins—B_1, B_2, and B_3—are produced by *Fusarium* spp. that grow on corn, most importantly *F. moniliforme*. In horses, relatively low exposures to fumonisins have been shown to cause equine leukoencephalomalacia, a disorder characterized by brain hemorrhage and necrosis, followed by death. Horses may also suffer liver damage and possibly a degree of kidney damage following dietary exposure to fumonisins. Liver and kidney effects are more pronounced in other species such as rodents, sheep, and rabbits. In swine, high doses of exposure to fumonisins seem to especially affect the lungs, leading to porcine pulmonary edema, a fatal condition in which fluid collects in the lungs. Low exposures result in reduced feed consumption. Early research in animals showed that fumonisins are potent cancer promoters and potentially weak initiators. The National Toxicology Program, along with the FDA's National Center for Toxicological Research in Jefferson, Arkansas, and Center for Food Safety and Applied Nutrition in Washington, DC, released the results of a massive study of the toxicity of fumonisin B_1 in May 1999. Their data showed that fumonisins are carcinogenic in rodents, although response differed by species and sex: male rats fed fumonisin B_1 developed liver and kidney cancers, while female mice developed liver cancer.

With regard to human toxicity, epidemiological data from southern Africa and China suggest a strong link between dietary fumonisin exposure and esophageal cancer. Human epidemiological studies, however, are not definitive, says Ken Voss, a research pharmacologist at the USDA Toxicology and Mycotoxin Research Unit in Athens, Georgia. "There are suggestive data that the fungus and the fumonisins are associated with esophageal cancer," he says. "But there are enough confounding dietary and environmental factors that the correlation, although tantalizing and suggestive, is as yet far from being proven." According to Voss, it has been shown that fumonisins have measurable and repeatable toxic effects in animal models.

Fumonisin toxicity seems to be mediated through inhibition of ceramide synthase, a key enzyme in the sphingolipid biosynthetic pathway. "To put it in layman's terms," says Voss, "the entire metabolism of sphingolipids in the cell is disrupted." The potential ramifications of this disruption can be far-reaching, he says. Until 15–20 years ago, sphingolipids were considered as having a purely structural role in cells; however, sphingolipid molecules and their derivatives are now recognized as very biologically active compounds. These compounds, says Voss, either initiate or act as messengers for many life-or-death decisions that the cell has to make. Such decisions include whether the cell embarks on apoptosis (cell death) or enters the cell cycle and replicates. From this point, he says, there are a host of potential steps and intermediaries leading to toxigenic or carcinogenic events.

Fusarium and Its Mycotoxins

In addition to fumonisins, *Fusarium* spp. produce several other mycotoxins. *F. graminearum* and *F. culmorum*, molds that contaminate corn, barley, wheat, and other crops, are capable of producing the toxins zearalenone and deoxynivalenol (also called vomitoxin). Different toxigenic species of *Fusarium* grow under different sets of climatic conditions. "The production of these compounds depends on a number of different conditions," says Retha Newbold, a supervisory research biologist at the NIEHS. "Just to have a product that is contaminated with mold is not to assume that mycotoxin is present. The mold may be there, but it may produce different levels of mycotoxins, or even different mycotoxins, depending on . . . different conditions."

Although zearalenone has low acute toxicity, it exhibits marked estrogenic effects in some species. Zearalenone and its metabolites, particularly α-zearalenol and β-zearalenol, have been shown to bind to estrogen receptors in experimental systems. Their estrogenic potential seems to fall between that of the endocrine-disrupting organochlorine pesticides and the more estrogenic compound diethylstilbestrol. Newbold indicates that the estrogenicity of zearalenone and its metabolites differs depending on the tissue and the species. For example, swine are especially sensitive and experience hyperestrogenism leading to reproductive problems and infertility following dietary zearalenone exposures. Other species such as cattle and sheep seem more resistant to zearalenone but may still experience some incidence of infertility, decreased milk production, and spontaneous abortion after ingesting high doses. Still other species, particularly chickens, appear even less sensitive.

It has been demonstrated that zearalenone and its metabolites may cause carcinogenesis or teratogenesis in some species, but further research is needed. Further research is also needed with regard to human toxicity. Currently, the International Agency for Research on Cancer classifies zearalenone as a 2A carcinogen, the highest possible classification when categorical human epidemiology is absent. Several countries have already established maximum allowable concentrations of zearalenone in food ranging from 0 to 1,000 micrograms per kilogram. Data on human toxicity are strongest with regard to estrogenic effects. For example, zearalenone was considered a possible etiological agent for precocious pubertal changes that were observed among Puerto Rican children for several years beginning in 1979. Thousands of children, some of whom were shown to have zearalenone or its derivatives in their blood, reportedly experienced symptoms. However, as other estrogens (phytoestrogens or residues of animal growth promoters) were potentially present in the children's diets, this outbreak might have stronger implications with regard to zearalenone's contribution to

the total environmental estrogen burden. Recent investigations using *in vitro* systems bolster the idea that zearalenone interacts with human estrogen receptors. For example, Craig Dees, a scientist in the Health Sciences Research Division of the Oak Ridge National Laboratory in Tennessee, and colleagues reported that zearalenone stimulates estrogen-receptor human breast cancer cells to enter the cell cycle *in vitro* [*EHP* 105(suppl 3):633–636 (1997)].

"For people who are studying endocrine disruptors and who are actually looking for some of the potential health effects during development, this [mycotoxin] is one of their concerns," says Newbold. "But I certainly don't think it has received the attention for human health that it should have in the United States."

Fusarium moniliforme
Photo: Anthony De Lucca
ARS/USDA

With regard to research, zearalenone seems to be overshadowed by deoxynivalenol, a more demonstrably toxic *Fusarium* metabolite. This mycotoxin has been linked to large-scale poisonings, human disease, and animal production problems throughout the world. Deoxynivalenol is one of the most common mycotoxins contaminating grains. It belongs to a class of compounds called trichothecenes, to which several other mycotoxins belong. Although deoxynivalenol is the least toxic of the trichothecenes, its toxicity is still substantial in both animals and humans. In large enough acute doses, it causes nausea, vomiting, and diarrhea and destroys blood cells. Animals, particularly pigs, demonstrate feed refusal and weight loss at lower doses. Deoxynivalenol has also been shown to have immunological effects in animal models. For example, research reviewed by James J. Pestka, a professor of food science and human nutrition at Michigan State University in East Lansing, and colleagues in the May 1996 issue of the *Journal of Toxicology and Environmental Health* demonstrates that deoxynivalenol interferes with normal immune system functioning in mice. They concluded that deoxynivalenol induces cytokines, immune system factors that help direct an inflammatory response. In the model they reviewed, mice exposed to deoxynivalenol developed symptoms similar to human IgA nephropathy, a kidney disorder characterized by inflammation.

Expanding Frontiers in Mycotoxicology

Trichothecenes may also be at the root of an outbreak of idiopathic pulmonary hemorrhage among infants in Cleveland, Ohio [*EHP* 107(suppl 3): 495–499 (1999)]. Among infants, pulmonary hemorrhage, or episodes of bleeding in the lungs, can arise from several causes such as injury or some forms of pneumonia. Unexplained, ongoing episodes of bleeding, as seen with the infant patients in Cleveland, is much rarer. Between 1993 and 1998, physicians at the Rainbow Babies & Children's Hospital in Cleveland saw 37 cases of pulmonary hemorrhage among infant patients; in the preceding 10 years, only three such cases had been encountered. Nearly all of the 37 infants were brought to the hospital because of breathing difficulties and required intensive care and ventilator support. Pulmonary hemorrhage wasn't always apparent before breathing difficulties surfaced but was detected once respiratory support began. Researchers suspected that some element in the infants' home environments was responsible for the symptoms because in several cases symptoms recurred when an infant returned to his or her home.

In a case–control investigation begun in November 1994 by the Centers for Disease Control and Prevention, researchers found evidence that the geographically clustered infants' homes were contaminated with *S. chartarum*, a mold not commonly found in home environments. *S. chartarum* is known to produce several trichothecenes, specifically satratoxins and roridin, as well as phenylspirodrimanes, cyclosporin, and a newly discovered class of compounds, the stachybocins. Researchers hypothesized that the mycotoxins, in combination with other stressors in the infants' environments such as tobacco smoke, caused the respiratory ailment that claimed the lives of 12 of the 37 infants. "The primary problem with indoor molds is that the health hazard is predominantly linked to people who are atopic—that is, they tend to be allergic," says Dorr Dearborn, an associate professor of pediatrics and biochemistry at the Case Western Reserve University School of Medicine in Cleveland and one of the researchers associated with the ongoing *S. chartarum* investigation. Says Dearborn, "What we're beginning to realize more recently, which is not really well known in the medical field, is that there are [indoor] molds—not just *Stachybotrys*, but probably a larger list of them—that produce mycotoxins that can have direct effects on health. This is still an area of both speculation/conjecture and some knowledge, but it's an area of active concern and with some research at least starting to be generated."

The precise mechanisms of the *Stachybotrys* mycotoxins are unknown, explains Dearborn. The trichothecenes may be able to trigger or aggravate an allergy problem directly, he says, but not through a traditional immunoglobulin E (IgE) pathway. Typically, an allergic response involves production of antibodies constructed from IgE against the allergen and some form of inflammation (such as asthma). Pestka's work to elucidate how deoxynivalenol induces nephropathy has shown that, at low levels, the trichothecene induces inflammatory mediators. This suggests a mechanism by which mycotoxins, including those produced by *Stachybotrys*, may produce airway disease or skin reactions without going through the typical IgE mechanisms, says Dearborn.

Further information on *Stachybotrys* toxicity is gleaned from older literature describing agricultural exposures to the mold in Eastern Europe and northern Russia during the 1940s and 1950s. In this literature, the effects include bleeding in the nose and throat (although not the lungs), skin irritation, and altered white blood cell counts. "The cellular mechanism of trichothecenes is well established: they are potent protein synthesis inhibitors," says Dearborn. Inhibition occurs via a single binding site on the ribosome, the cellular location of protein construction. Depending on the specific trichothecene, construction breaks down during its initiation, elongation, or termination stages, he explains. However, he continues, the details behind their effects on the immune system remain unknown.

Dearborn and his associates are currently in the early stages of a five-year grant from the NIEHS to develop an infant model for *S. chartarum* exposure as an outgrowth of their investigations into the Cleveland outbreak of idiopathic pulmonary hemorrhage. "In the Cleveland situation we're dealing with an epidemiological association—that is, we've found [the mold] in the houses of the cases more than we found it in the control houses. The link is not absolute at all; it's simply an epidemiological link," says Dearborn. One of the scientists' immediate challenges comes in the form of identifying a biomarker for exposure, a difficult task given how rapidly the suspected toxins are metabolized. Finding a biomarker is difficult

because suspected *Stachybotrys* toxins are rapidly metabolized and most people do not form antibodies in response to the mold. The researchers' early experiments to duplicate the disorder in infant animals have been promising. "What we have shown is that if the spores of *Stachybotrys* are instilled in the tracheas of young rat pups, they will develop pulmonary hemorrhage. Initial results suggest that we are on the proper route to develop an infant model for the disorder," says Dearborn.

Areas for Further Research

The diverse spectrum of mycotoxins produced by *S. chartarum* illustrates one of the more vexing issues in mycotoxicology: mycotoxins usually occur in mixtures. As a result, researchers recognize that interactions are possible although they are difficult to characterize. There is particular interest in exploring potential synergies, or interactions in which exposure to more than one mycotoxin results in a multiplication, rather than an addition, of risks. "The kinds of experiments that are necessary to elucidate the nature of synergy are complicated," says J. David Miller, a professor of chemistry at Carleton University in Ottawa, Canada, who recognized early on that the toxins produced by molds are typically mixtures of toxins. Such experiments would require a lot of resources that currently are not available, so investigating potential additive effects or synergy between mycotoxins—or between mycotoxins and other environmental factors—is, for the most part, not a major research focus. Nevertheless, researchers have commented, for example, on the potential interaction between fumonisins and aflatoxin. For now, says Miller, the most important synergy that has been investigated with regard to mycotoxins is the one that exists between aflatoxin and hepatitis B.

Another area of mycotoxin research that seems ripe for further investigation concerns defining the subtler effects of individual mycotoxins. In the area of veterinary toxicology, pinning down information on such effects is an especially active area of interest, according to George Rottinghaus, a chemist in the toxicology section of the University of Missouri Veterinary Medical Diagnostic Laboratory in Columbia. "Those are what I call the gray areas," he says. "Everybody's done acute, subacute, and that type of work, but it takes a lot more effort to get into these more subtle changes. [With] a lot of these subtle things, you really wouldn't have symptoms. It would be more of a performance- or immune-type response effect that most people wouldn't see." He offers an example: "All of a sudden your animals might be sicker than they normally are . . . or they [would be] off 5–10% in milk production, or they don't gain [weight] quite the way they were supposed to." Rottinghaus points out that these symptoms can be attached to many other factors, but it's hard to pin them to either mycotoxins or alternative explanations.

Controlling Exposures and Mitigating Effects

The best means of preventing the health effects of mycotoxins is to prevent exposure—a task more easily noted than achieved. In the agricultural arena, postharvest control of storage fungi is handled through proper drying and storing of grains. These measures are accomplished with varying degrees of adequacy depending on the available equipment, storage facilities, and other variables. Success in preventing field contamination can be even more variable owing to factors such as insect infestation, drought, or weather events such as hail storms. Once crops are damaged, an opening appears for fungal contamination. Whether or not fungi will exploit that opening depends on other factors, including the prevailing temperature, humidity, and water content. In some fields, microclimates may exist so that one part of the field can be heavily contaminated while neighboring sections are completely untouched.

Although use of antifungal agents and other chemicals is potentially effective, researchers are investigating several strategies that don't rely on chemical applications. "A number of the corn companies are trying to develop hybrids that are resistant to the *Fusarium* infection," says Rottinghaus. According to Voss, researchers at the USDA are exploring another avenue in investigations centering on potential biological controls of fungal growth and toxin production in corn plants. The idea behind their strategy is to use nontoxigenic bacterial or fungal species as bioexclusion agents that would outcompete fungi in the field and in storage. This technique would only be used for corn destined for animal feed, and Voss indicates that USDA researchers anticipate some commercial applications of the technique within the next five years.

In some areas of the world, fungal control techniques are more urgently needed. Miller points out that in North America the population experiences a relatively low risk from mycotoxins owing to a diverse diet and the range of zones in which crops are raised. "In North America, we produce large amounts of a crop, and we only use a small percentage of it for human food. If we have a bad year in Texas, it's unlikely it's [also] a bad year in Iowa. We have the luxury, by and large, to pick and choose in terms of excluding crops from our food system if it's necessary," he explains. For example, in 1996 wheat grown in Michigan, Maine, and Ontario couldn't be used because it was heavily contaminated with vomitoxin. However, because the commodity is also grown, albeit in lesser quantities, in Alberta and the U.S. Pacific Northwest, buyers could find supplies elsewhere. "In developing countries, that luxury is not there," Miller says.

Stachybotrys chartarum
Photo: Dorr Dearborn

In recognition of this fact, researchers are attempting to find other means of protecting populations from the health effects associated with mycotoxin exposure. Much effort has been devoted to applying such measures in AFB_1-exposed populations. One technique has been to promote vaccinations for hepatitis B in areas with high AFB_1 exposures. Another strategy explores the potential for altering the metabolism of the toxic compounds. Recently, human trials were conducted with oltipraz, a compound that interferes with the mechanism of action of aflatoxin. The trials were published in the February 1999 issue of the *Journal of the National Cancer Institute*. "We'd like to believe from the data we have from the oltipraz clinical trial that agents that can blunt the metabolism of aflatoxin are certainly going to be important in terms of preventing aflatoxin-mediated DNA damage," says Groopman, who collaborated with researchers in China on this study. "With the oltipraz intervention, we found that we can modulate the metabolism of aflatoxins in people and shunt the metabolism toward non–DNA-damaging species. If that can be replicated by other dietary agents, that is probably going to be a very important way of intervening in large populations," he concludes.

Efforts to control exposure to mycotoxins are certainly better today than in ancient times, but they still are not perfect. For example, the testing for mycotoxins such as aflatoxin only involves grains that enter interstate commerce and thus doesn't protect people who might consume highly contaminated locally grown crops. Other populations may face greater risk simply because they consume higher-than-average amounts of certain commodities or because they live or work in poorly maintained buildings. Finally, research has primarily emphasized dietary routes of exposure. Knowledge about the long-term effects of other exposures is lacking. "The toxins that enter the crops we use for food . . . [are] sort of a by-product of chemical warfare that's going on at a microbiological level," says Miller. There are lots of experiments to do, he muses, and not a lot of resources to do them.

Julia R. Barrett